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THE EFFECTS OF CATHEPTIC ENZYMES ON MUSCLE PROTEINS,(U)
JUN 80 J E WALKER, F M ROBBINS, S H COHEN

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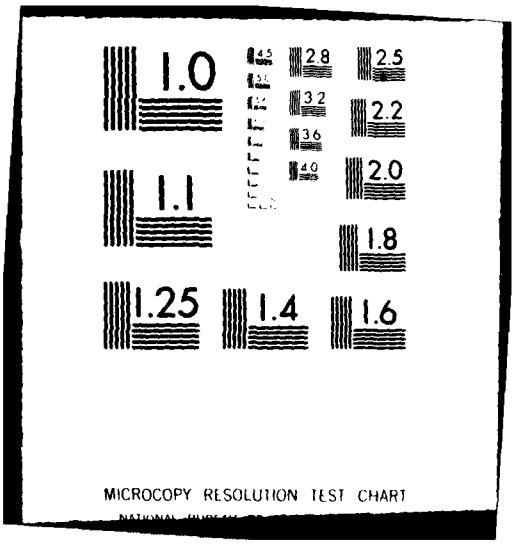
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MICROCOPY RESOLUTION TEST CHART

*WALKER, ROBBINS & COHEN

THE EFFECTS OF CATHEPTIC ENZYMES ON MUSCLE PROTEINS

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JUN 80

It is well recognized that two structural components-collagen of the connective tissue and the actomysin complex of the contractile apparatus determine the tenderness of meat. Muscle collagen is comprised of three genetically distinct types, each stabilized by lysine derived cross-links. As the animal matures the degree of cross-linkage increases relating to the textural alteration in the meat from older animals. Textural changes not related to the age of the animal occur postmortem (after slaughter) in which the meat goes through a cycle of toughening (rigor) and tenderization. The natural tenderization following rigor occurs after 4 or 5 days postmortem and involves primarily changes in the interaction of two major elements of the contractile apparatus; myosin (the thick filament) and actin (the thin filament). Examination of the changes in the ultrastructure of the muscle during the natural tenderization process include: loss of the "Z" band (a structure occuring periodically along the muscle fiber to which it is believed attaches thin filaments to each other), and changes in the distances between the thick and thin filaments. In 1973 Eino and Stanley (1) showed that a group of proteases in muscle produced structural alterations in muscle resembling those which occured during postmortem tenderization. These enzymes were released maximally after 5 days postmortem. The group of enzymes are known to be acid hydrolases called cathepsins and are found within special organalles in the muscle called lysosomes. In 1976 Robbins and Cohen (2) of the Food Sciences Laboratory of NARADCOM, reported that muscle when treated with an extract of bovine spleen could be considered as an exogenous tenderizer which would simulate the natural aging process that occurs postmortem.

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The work that I will report on today is the progress since the 1976 paper. This work gives biochemical, ultrastructural and technological evidence for the use of spleen enzymes (cathepsins) as exogenous tenderizing agents. We have recently published these findings (3).

The extraction of the active enzyme fraction is shown in figure 1. The release of the catheptic enzymes from the lysosomes occurs when the pH is lowered to 3.5. The subsequent lyophilate is rehydrated prior to its application. The catheptic enzymes found in the spleen extract are given in figure 2. ~~Our~~ attention has been focused on cathepsin D and B. As a model system of meat in order to examine the effect of these catheptic enzymes we chose bovine myofibrils. Myofibrils contain thick (myosin) and thin (actin) filaments as well as ~~the regulatory proteins~~ (tropomyosin and the troponins). In addition the myofibril contains a protein (actinin) which many consider the main component of the "Z" band. The effect of the action of the spleen enzymes was followed by electrophoretic analyses of the treated myofibrils. ~~In these experiments~~ the enzyme extract was added to a solution of myofibrils and after ~~the~~ specified incubation time the myofibrils were solubilized in a phosphate buffer ~~containing~~ urea, sodium dodecylsulfate, and dithiothreitol (a reducing agent). The electrophoresis was performed on polyacrylamide gels and the individual proteins were visualized by staining with coumassie blue dye. The results of the reaction with the spleen extract is shown in figure 3, where A is the control myofibril ~~untreated~~, B and C are myofibrils treated with spleen enzyme (cathepsin D) and with cathepsin D (isolated from muscle) respectively. The most dramatic change is seen in the myosin which has decreased in molecular weight from 200,000 daltons to 145,000 daltons, other changes occured in the regulatory proteins especially troponin I and troponin C. Changes in myosin might be expected to weaken the structure of the muscle fiber and lead to an alteration in tensile strength (a tenderization. We compared the action of the spleen enzyme to a commercial meat tenderizer, ~~papain~~ which is derived from plants and goes under the trade name of "Adolph's Tenderizer". The myofibrils were treated with papain in a similar manner as the spleen enzyme and analyzed by gel electrophoresis. The results are shown in figure 4. The control (untreated) myofibril is A and the papain treated, B. The action of papain leads to a complete hydrolysis of the myofibrilar proteins. Such an extensive proteolysis may be the cause of the over tenderizing action observed in the indiscriminate use of commercial meat tenderizers. In order to examine the extent to which the organization of the myofibril is disrupted on the molecular level during the reaction with the spleen enzymes, a series of experiments involving electron spin resonance were performed. In these experiments low molecular weight organic compounds which posses an unpaired electron in one region of their structure was covalently

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attached to the myofibril through a functional group in another region of their structure. The two organic compounds we used in our studies are shown in figure 5A. They attached themselves primarily to one region of the myosin (thick filament). The attached organic free radicals acted as reporter groups in the myofibril since their electroparamagnetic resonance spectra (figure 5B) would be altered in shape if there were structural alterations occuring near to them. In figure 6 is shown the effect of 4 commercial meat tenderizers A,B,C, and D on myofibrils containing the free radical reporter groups. Their effect is compared to the action of the spleen enzymes (E and F). The parameter "R" on the ordinate scale represents the degree of structureal alteration in the area near the reporter groups. We noted that each of the commercial tenderizers bring about rapid structural disorder while the spleen enzyme treated samples showed no such effect. Ultrastructural examination on the spleen enzyme treated myofibrils was made by scanning electron microscopy. After treatment with the spleen enzyme the myofibrils were washed and fixed in glutaraldehyde. Following a dehydration procedure, the samples were critically point dried with CO_2, mounted and coated with gold-palladium. The prepared specimens were examined in a Coates and Welter Model 100-Z Field Emission Scanning Electron Microscope. Figure 7 shows the S.E.M. micrographs of the untreated (A) and the enzyme treated myofibril (B and C). The structural alterations seen in the enzyme treated myofibrils are; a loss of the "Z" band structure (refer to the arrow in the figure), and a shortening of the lengths of the sarcomeres (the region of the myofibril bounded by each "Z" band). Loss of the "Z" band is the commonly observed ultrastructural change that postmortem meat undergoes during the natural tenderization process. In the natural tenderization process this "Z" band loss occurs after 4 to 5 days whereas the spleen enzyme extract brings this about after one hour.

Although our biochemical and ultrastructural experiments had shown that the changes brought about by the spleen enzymes were very similar to those reported during natural tenderization, a technological experiment was needed to prove its potential usefulness for the Army;s Food Program. In this experiment, precooked freeze-dehydrated beef slices were placed in vacuum sealed cans and stored at room temperature. At periods of 3,6,9, and 12 months dehydrated beef slices were rehydrated in water with and without the spleen enzyme extract. Figure 8 shows a textural measurement on the rehydrated meat slices performed on a Instron Universal Testing Instrument. At all times during the storage experiment the effect of the spleen enzyme was to increase the tenderness of the beef slices.

The work of the Food Sciences Laboratory of NARADCOM has thus shown that extracts from bovine spleen, highly active in catheptic enzymes have an excellent potential for use as exogenous meat

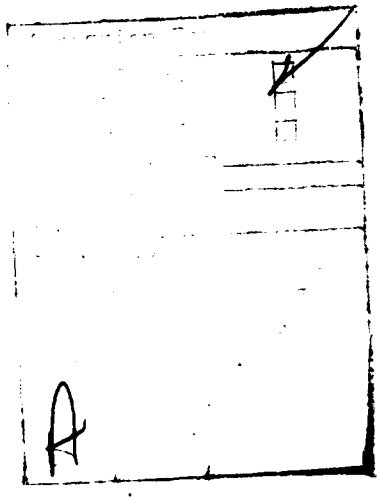
tenderizers because of their selectivity of action and ready availability from bovine spleen which is a cheap by-product of the meat industry.

REFERENCES:

1. Eino, M.F. and Stanley, D.W. 1973. Catheptic activity, textural properties and surface ultrastructure of postmortem beef muscle. J. Food Sci. 38:45.

2. Robbins, F.M. and Cohen, S.H. 1976. Effects of Catheptic enzymes from spleen on the microstructure of bovine semimembranosus muscle. J. Text. Stud. 7:137.

3. Robbins, F.M., Walker, J.R., Cohen, S.H., and Chatterjee, S. 1979. Action of proteolytic enzymes on bovine myofibrils. J. Food Sci. 44:1672.



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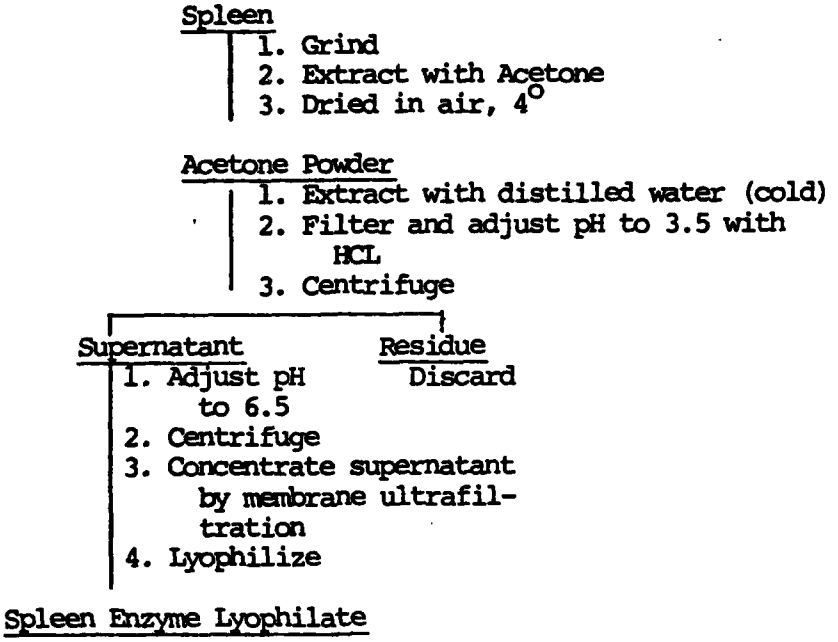

FIGURE 1

(377)

LYSOSOMAL CATHEPTIC ENZYMES

1. Cathepsin A - Carboxypeptidase, has little action on protein itself, but acts synergistically with endopeptidases
2. Cathepsin B - Endopeptidase with broad specificity similar to papain
3. Cathepsin C - Exopeptidase capable of sequentially splitting dipeptides from the amino terminals of polypeptide chains.
4. Cathepsin D - Endopeptidase that resembles pepsin in its specificity

FIGURE 2

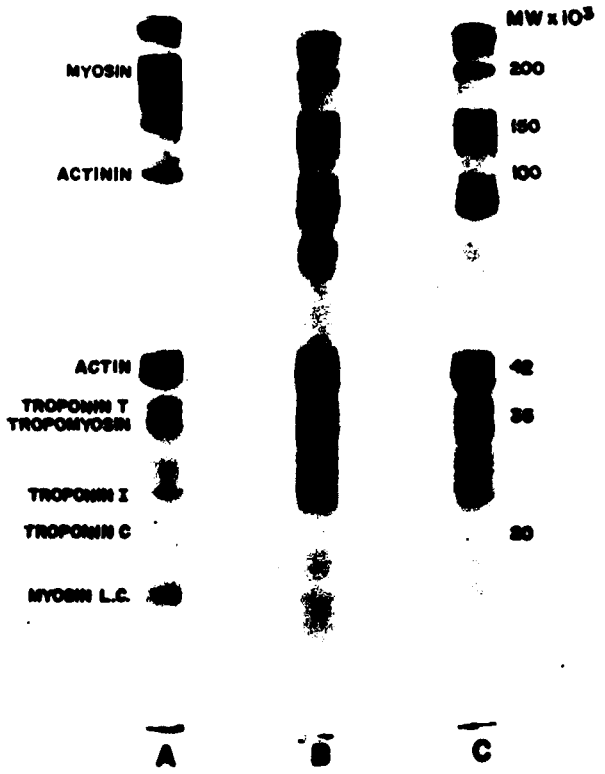
MW x 10³
200
150
100
MYOSIN
ACTININ
ACTIN
TROPONIN T
TROPOMYOSIN
TROPONIN I
TROPONIN C
MYOSIN L.C.
42
36
20
A
B
C

FIGURE 3

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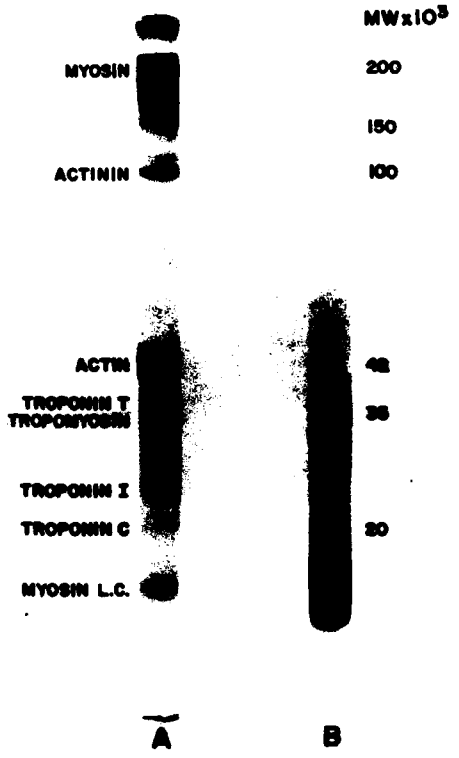
MW x 10³
MYOSIN
200
150
ACTININ
100
ACTIN
42
TROPONIN T
TROPOMYOSIN
36
TROPONIN I
TROPONIN C
20
MYOSIN L.C.
A
B

FIGURE 4

380

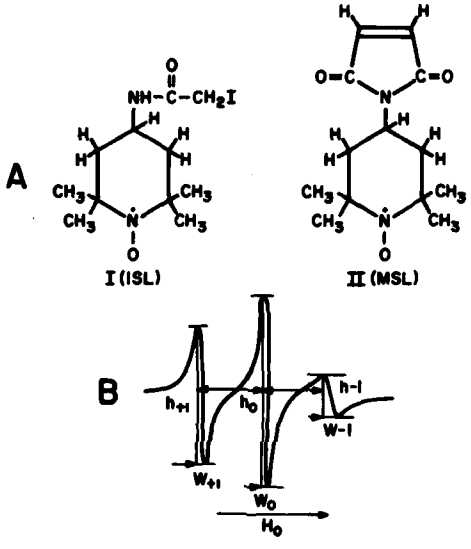

FIGURE 5

381

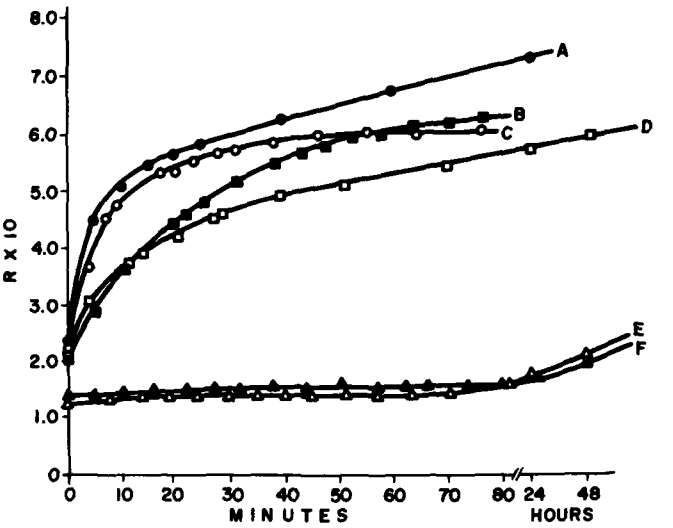
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
R X 10
A
B
C
D
E
F
0 10 20 30 40 50 60 70 80 24 48
MINUTES
HOURS

FIGURE 6

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FIGURE 7

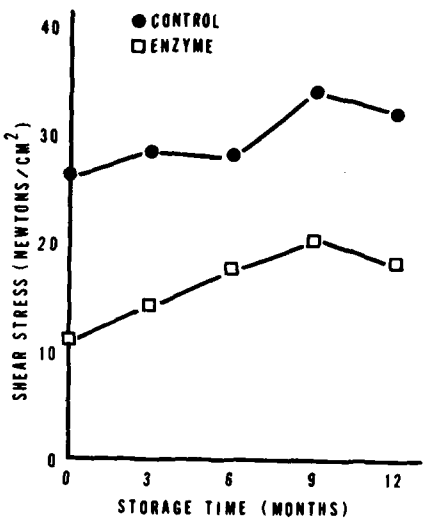
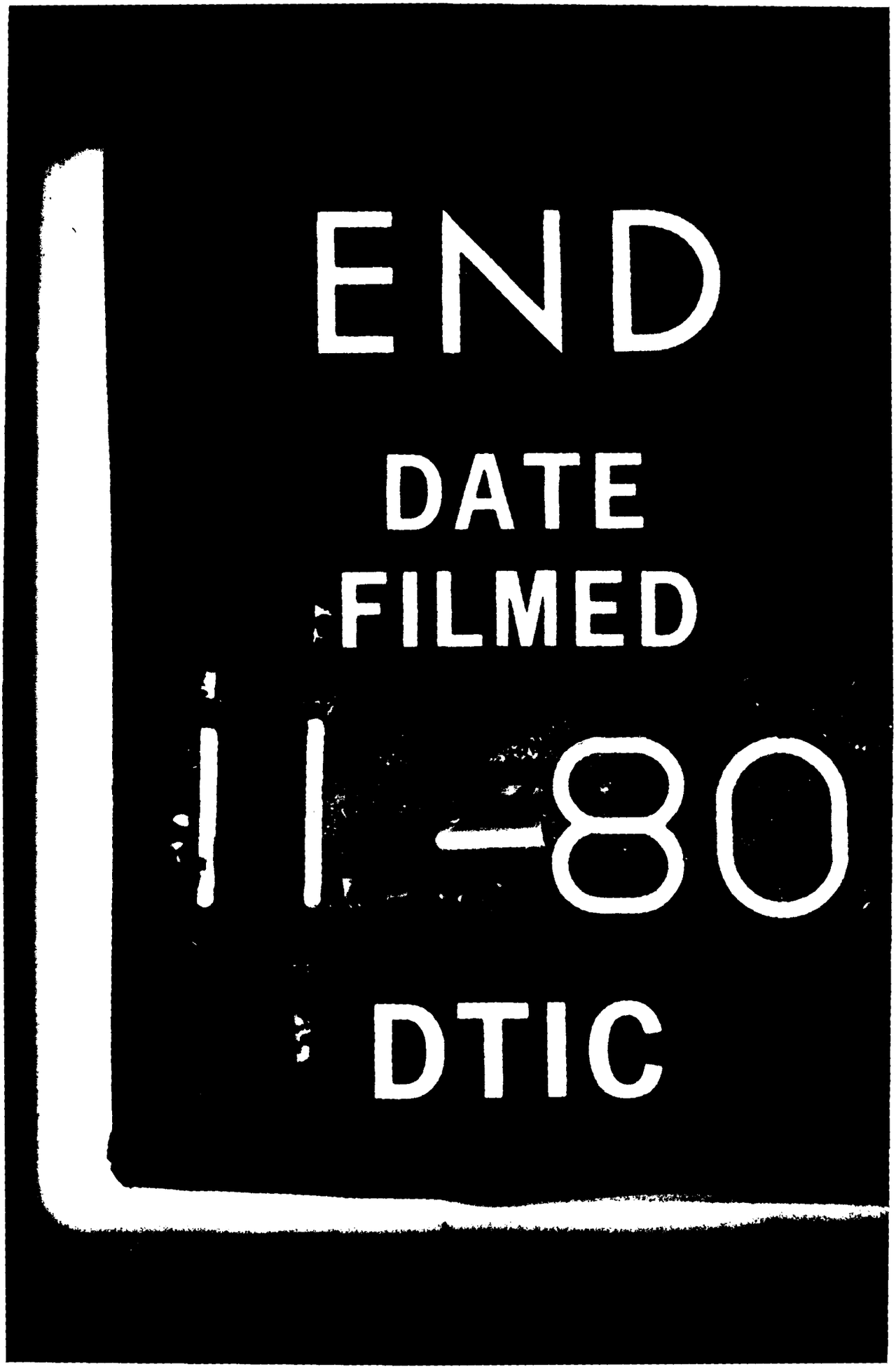

CONTROL
ENZYME
SHEAR STRESS (NEWTONS/CM²)
40
30
20
10
0
0
3
6
9
12
STORAGE TIME (MONTHS)

FIGURE 8

(384)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